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Modulation of the actions of tyrosine by α_2-adrenoceptor blockade

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1 Eight normal subjects were given, in double-blind, random order L-tyrosine 50, 250 and 500 mg kg^{-1} and placebo orally. Plasma tyrosine concentrations rose in a dose-dependent manner, without affecting the concentrations of the other large neutral amino acids. Tyrosine stimulated the secretion of prolactin and thyrotrophin (TSH) but had no effect on the plasma concentrations of adrenocorticotrophic hormone (ACTH), cortisol, growth hormone or the gonadotrophins.

2 The lack of a stimulant effect of tyrosine on ACTH secretion was presumed to be due to activation of one of the negative feedback mechanisms that control the rate of synthesis and release of the catecholamines, and this hypothesis was tested by examining the effects of the α_2-adrenoceptor antagonist idazoxan on the actions of tyrosine.

3 Seven normal males were given on 6 separate occasions tyrosine 250 and 500 mg kg^{-1} and placebo orally following pretreatment with saline and idazoxan (0.1 mg kg^{-1} i.v.). Following pretreatment with idazoxan, tyrosine stimulated the secretion of ACTH and noradrenaline in a dose-dependent manner, although neither tyrosine nor idazoxan on their own had any effect on the secretion of either substance.

4 The lack of effect of tyrosine when given on its own appears to be due, partly, to activation of α_2-adrenoceptors, which inhibit the release of noradrenaline. Idazoxan caused a small increase in systolic blood pressure, both when given on its own and in combination with tyrosine. Neither tyrosine nor idazoxan had any significant effect on the state of behavioural arousal, as measured by visual analogue scales, or on the secretion of growth hormone or the gonadotrophins.

Introduction

Our previous investigations of the effects of the catecholamines on the secretion of adrenocorticotrophic hormone (ACTH) in man demonstrated that intravenous infusion of the α_1-adrenoceptor agonist methoxamine stimulated the secretion of ACTH in a dose-dependent manner and the effect was blocked by concomitant administration of the α_1- adrenoceptor antagonist thymoxamine (Al-Damluji *et al.*, 1987a). The site of action of these stimulant α_1-adrenoceptors appears to be in the central nervous system; the effects of methoxamine are not reproduced by equipotent doses of the more hydrophilic α_1-adrenoceptor agonist noradrenaline, which reaches the pituitary gland and the median eminence following a systemic injection, but does not cross the blood-brain barrier. Further studies demonstrated that the α_2- and β-adrenoceptor agonist properties of noradrenaline did not account for the differences from methoxamine (Al-Damluji *et al.*, 1987a). Intravenous infusions of adrenaline had no enhancing effect on the activity of synthetic ovine corticotrophin releasing factor in man (Al-Damluji *et al.*, 1987b), in contrast to the findings in cultured rat adenohypophysial cells *in vitro* (Vale *et al.*, 1983; Giguere & Labrie, 1983). The physiological significance of the stimulant α_1-adrenoceptor mechanism on ACTH secretion was demonstrated in 2 situations: the cortisol secretory pattern during waking hours and the ACTH and cortisol secretory responses to food ingestion were both enhanced by intravenous infusions of the α_1-adrenoceptor agonist

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methoxamine and reduced by the α_1-adrenoceptor antagonist thymoxamine, suggesting that ACTH secretion in these two physiological circumstances is mediated by α_1-adrenoceptors (Al-Damluji *et al.*, 1987c,d).

In our current studies, we have been examining the effects of endogenous catecholamines on the hypothalamo-pituitary adrenal axis in man. The aims of this study were: (1) to investigate whether the plasma ACTH response to food ingestion is caused by absorption of the catecholamine precursor and dietary amino acid tyrosine, and its subsequent conversion to noradrenaline. This has been done by administering a dose of tyrosine that is similar to the estimated tyrosine content of a standard meal previously shown to stimulate ACTH secretion. (2) To examine the effects on anterior pituitary hormone secretion of supraphysiological doses of tyrosine. (3) To examine the role of α_2-adrenoceptors in modulating the actions of tyrosine. The synthesis and release of noradrenaline is controlled by at least two negative feedback processes. Firstly, the activity of the enzyme tyrosine hydroxylase is inhibited by the products of the catecholamine synthesis pathway (Nagatsu *et al.*, 1964; Udenfriend *et al.*, 1965). As tyrosine hydroxylase is the rate limiting step in the synthesis of catecholamines, this forms a negative feedback process in the rate of conversion of tyrosine to noradrenaline. Secondly, noradrenaline released from the nerve terminals activates presynaptic α_2-adrenoceptors which limit the further release of neuronal noradrenaline (Langer, 1977). We have examined the possible role of this latter mechanism in modulating the responsiveness to tyrosine, by examining the effects of the α_2-adrenoceptor antagonist idazoxan on the hormonal, behavioural and cardiovascular effects of tyrosine.

Methods

Tyrosine dose-response study

Eight normal subjects (including 4 females in the follicular phase of their regular menstrual cycles) were studied following an overnight fast on four occasions each, starting at 10 h 00 min, following insertion of an intravenous cannula one hour earlier. They received, in double-blind, random order L-tyrosine 50, 250 and 500 mg kg^{-1} and placebo (calcium carbonate) as an emulsion in 150 ml of water orally. The 50 mg kg^{-1} dose of tyrosine is similar to the estimated tyrosine content of a standard meal which had previously been shown to stimulate ACTH and cortisol secretion in normal subjects (Al-Damluji *et al.*, 1987d). The subjects were studied in the recumbent position in a quiet room and blood samples were taken at 15 min intervals.

Interaction of tyrosine and idazoxan

Seven normal male subjects were studied on 6 occasions each, receiving: (1) idazoxan 0.1 mg kg^{-1} intravenously over 12.5 min, followed by tyrosine 250 mg kg^{-1} orally; (2) idazoxan followed by tyrosine 500 mg kg^{-1}; (3) 0.15 M saline intravenously followed by tyrosine 250 mg kg^{-1}; (4) 0.15 M saline followed by tyrosine 500 mg kg^{-1}; (5) idazoxan followed by placebo (calcium carbonate); (6) 0.15 M saline followed by placebo. Idazoxan was dissolved in 0.15 M saline 0.1 ml kg^{-1}, and the same volume of 0.15 M saline was administered as a control. Other details of the investigative procedure were as described above, except that the studies were carried out under electrocardiographic monitoring.

Heart rate and blood pressure (sphygmomanometry, phases 1 and 5) were measured at 15 min intervals. At 30 min intervals, the subjects were asked to assess their state of behavioural arousal on 10 centimetre visual analogue scales (awake-sleepy; energetic-lethargic; strong-weak; troubled-tranquil; quick witted-mentally slow; interested-bored; comfortable-uncomfortable). The subjects were asked to regard the line as the full range of each dimension. In preliminary investigations, the sensitivity of the visual analogue scales to physiological changes in behavioural arousal was assessed by administering them to 10 normal females at 23 h 00 min and at 11 h 00 min. There were statistically significant increases from 23 h 00 min to 11 h 00 min in the scales for 'awake' (34.1 ± 6.5 to 79.8 ± 4.6, mean $\pm$ s.e.mean, $P < 0.001$, Student's t test), 'energetic' (40.6 ± 4.4 to 72.8 ± 4.0, $P < 0.001$), and 'strong' (52.4 ± 2.7 to 68.9 ± 4.9, $P < 0.001$).

Plasma tyrosine concentrations were measured by high performance liquid chromatography (h.p.l.c.) followed by fluorimetric detection. Tyrosine was extracted from plasma using 3% salicyl sulphonic acid, pH 1.8. The apparatus for h.p.l.c. consisted of a Waters WISP 710B automatic injector, M45 h.p.l.c. pump, model 420 fluorescence detector, Data Module System Controller and 7 μm sulphonated polystyrene cation exchange h.p.l.c. column (15 x 0.46 cm). Norleucine was used as an internal standard and the mobile phase was 0.2 M trisodium citrate, pH 4.0. Post-column derivatisation was achieved with *o*-phthalaldehyde and 3-mercaptopropinic acid. The intra-assay coefficient of variation was 4.5%. The plasma concentrations of the other amino acids (except tryptophan) were measured with a Waters amino acid autoanalyser as above but eluted with a citrate gradient.

Plasma tryptophan concentrations were measured by h.p.l.c. followed by fluorimetric detection (Kratos FS970 fluorimeter) without derivatisation. Tryptophan was extracted from plasma with 5% trichlo-

roacetic acid, centrifuged, and the supernatant injected onto a 4.6 × 100 mm column of 3 micron ODS Hypersil. The mobile phase was 0.1 M sodium dihydrogenphosphate pH 4.0 and methanol in a ratio of 4:1. The excitation and emission wave lengths were 220 and 370 nm, respectively. The recovery of added tryptophan was 65–75% and the coefficients of variation of the assay were 3%.

Plasma ACTH concentrations were measured in unextracted plasma by a double antibody immunoradiometric assay (IRMA) using monoclonal antibodies (White *et al.*, 1987). In six consecutive assays, the lower limit of detection was $4.1 \pm 0.8\,ng\,l^{-1}$ and the coefficient of variation was 6.0% at $50\,ng\,l^{-1}$. Plasma cortisol concentrations were measured by radioimmunoassay (Al-Damluji *et al.*, 1987b). The lower limit of detection of this assay is $50\,nmol\,l^{-1}$ and the intraassay and interassay coefficients of variation are 4.5% and 8%, respectively. Plasma noradrenaline and adrenaline concentrations were measured by h.p.l.c. followed by electrochemical detection (Bouloux *et al.*, 1985). The lower limits of detection are $0.05\,nmol\,l^{-1}$ for both catecholamines and the coefficients of variation of the assay are less than 8% at physiological concentrations. Serum concentrations of prolactin, growth hormone, leutinizing hormone (LH), follicle stimulating hormone (FSH), thyroxine (T_4) and liothyronine (T_3) were measured by radioimmunoassays and thyrotrophin (TSH) by an IRMA using reagents supplied by The North East Thames Region Immunoassay Service. The coefficients of variation of these assays are less than 10%.

The data are expressed as the means ± s.e.mean. For statistical purposes, undetectable values were considered as the lower limit of detection of the assay. Statistical examination was by analysis of variance of changes in the area under the curve. In the tyrosine dose-response study, the baseline was the mean of the values at −15 and 0 min, and in the tyrosine-idazoxan interaction study, the baseline was the value at −15 min. The changes in the visual analogue scales were examined by analysis of variance for each time point after subtraction of the baseline value. Arc sine transformation was carried out as the data were not normally distributed.

The studies were approved by the Ethical Committee of St Bartholomew's Hospital and informed, written consent was obtained from all the subjects. The volumes of blood taken from each individual did not exceed 550 ml in total. Physical examination, electrocardiogram and a series of haematological and biochemical investigations were normal in all the subjects.

L-Tyrosine was purchased from Scientific Hospital Supplies, Liverpool and idazoxan was a gift from Reckitt & Colman plc, Kingston-Upon-Hull, U.K.

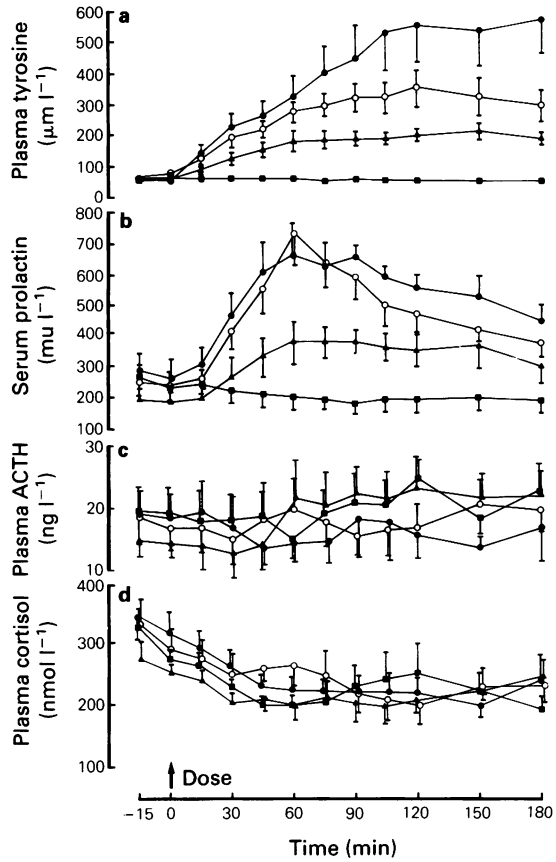

Figure 1 The effects of oral placebo (■) and tyrosine 50 (▲), 250 (○) and (●) $500\,mg\,kg^{-1}$ on circulating tyrosine (a), prolactin (b), ACTH (c) and cortisol (d) concentrations in eight normal subjects. Vertical lines indicate s.e.mean.

Results

Tyrosine dose-response study

Plasma tyrosine concentrations rose in a dose-dependent manner following the administration of tyrosine (Figure 1). Tyrosine had no effect on plasma ACTH, cortisol (Figure 1), LH, FSH or growth hormone concentrations (data not shown). Tyrosine stimulated the secretion of prolactin in a dose-dependent manner and a maximal effect was obtained with the $250\,mg\,kg^{-1}$ dose (Figures 1 and 2). Peak serum prolactin concentrations were reached at 60 to 90 min. All three doses of tyrosine partially prevented the circadian fall in plasma TSH concentrations seen after the placebo. The increments in serum prolactin concentrations in response to tyrosine were approximately 1000 times

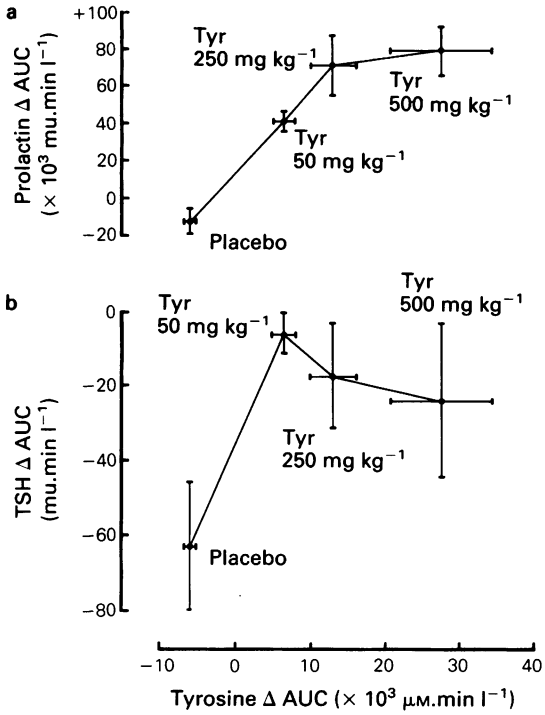

Figure 2 The relationship of tyrosine (Tyr) dose to serum prolactin (a) and thyrotrophin (TSH) responses (b), expressed as changes in the area under the curve (Δ AUC) for plasma tyrosine, serum prolactin and TSH concentrations. Note that the units for prolactin are 1000 times larger than for TSH.

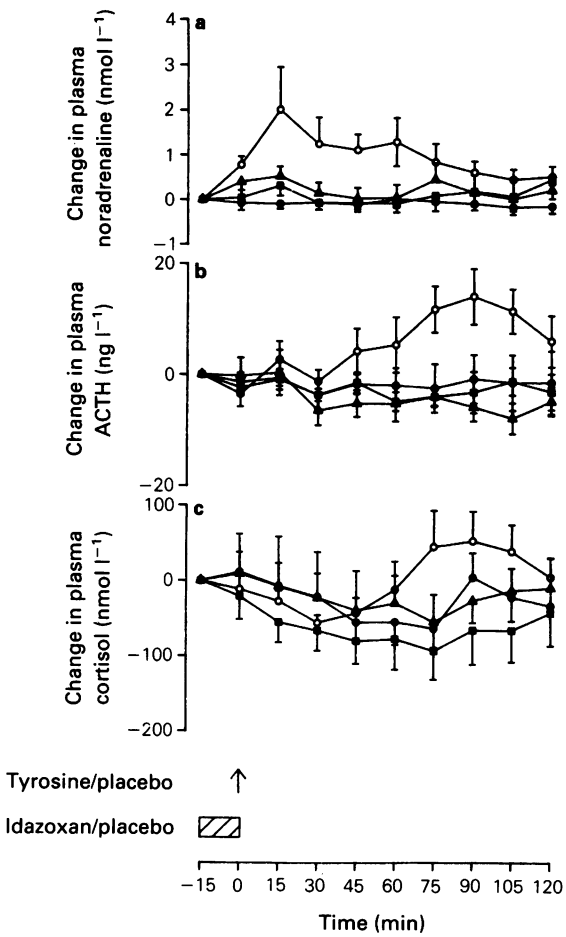

Figure 3 The effects of placebo (■), idazoxan (▲, 0.1 mg kg^{-1}), tyrosine (●, 500 mg kg^{-1}) and a combination of tyrosine and idazoxan (○) on plasma noradrenaline (a), ACTH (b) and cortisol (c) concentrations in seven normal subjects. Idazoxan was administered intravenously over 12.5 min starting at −15 min and tyrosine was administered orally at 0 min. Vertical lines indicate s.e.mean.

greater than those in serum TSH concentrations (Figure 2). There were no significant changes in serum T_4 or T_3 concentrations in comparison to placebo (data not shown). Tyrosine had no significant effect on blood pressure or the visual analogue scales in any of the doses (data not shown). No side effects or unpleasant sensations were reported.

Interaction of tyrosine and idazoxan

Idazoxan had no statistically signfiicant effect on plasma tyrosine concentrations, either when it was given alone or in combination with tyrosine. Idazoxan and tyrosine had no effect on plasma ACTH, cortisol or noradrenaline concentrations when they were administered on their own. In contrast, the combined administration of tyrosine and idazoxan caused dose-dependent increases of plasma ACTH, cortisol and noradrenaline concentrations in comparison to placebo (Figures 3 and 4). The increments in plasma ACTH and noradrenaline concentrations were statistically significant following the 500 mg kg^{-1} dose of tyrosine ($P < 0.02$ and $P < 0.01$, respectively), but the changes following the 250 mg kg^{-1} dose did not reach statistical significance. Idazoxan had no significant effect on adrenaline, prolactin, TSH, LH, FSH or GH concentrations and did not modify serum prolactin or TSH concentrations following the administration of either dose of tyrosine (data not shown). Idazoxan caused small but significant increases in systolic blood pressure in comparison to placebo, both when it was given alone and in combination with tyrosine ($P < 0.001$). The changes in systolic blood pressure (60 min

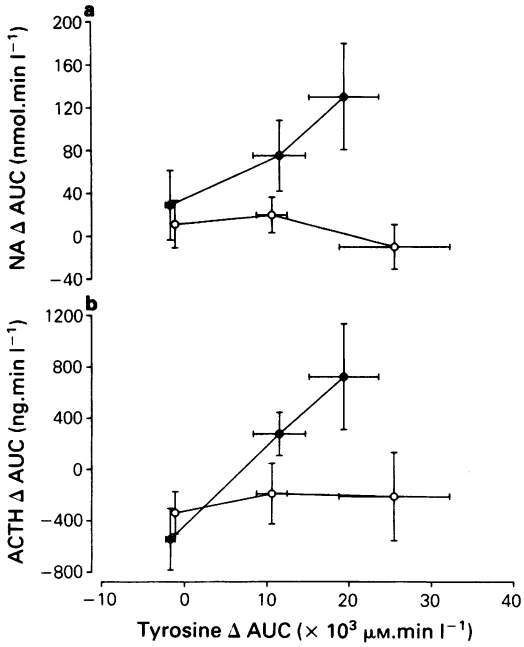

Figure 4 The relationship of tyrosine dose to plasma (a) noradrenaline (NA) and (b) ACTH responses, expressed as changes in the area under the curve (Δ AUC) for plasma tyrosine, noradrenaline and ACTH concentrations. (●) Pretreatment with idazoxan; (○) pretreatment with saline.

value − baseline, in mmHg) were: placebo: −1.7 ± 1.7; tyrosine 500 mg kg^{-1}: +2.3 ± 2.0; idazoxan: +7.3 ± 2.1; tyrosine 500 mg kg^{-1} + idazoxan: +10.7 ± 0.4. There were no significant changes in heart rate, diastolic blood pressure or any of the visual analogue scales following any drug (data not shown). No side effects or unpleasant sensations were encountered following any of the drugs.

The administration of tyrosine and idazoxan had no effect on the concentrations of the other large neutral amino acids (Table 1).

Discussion

We found that tyrosine had no stimulant action on ACTH or cortisol secretion in doses equivalent to those found in food. The ACTH response to feeding is therefore unlikely to be due simply to the ingestion of tyrosine and its subsequent conversion to catecholamines, and the mechanism of food-induced ACTH secretion remains unknown. Ishizuka *et al.* (1983) demonstrated that tyrosine had a small stimulant effect on the secretion of cortisol, but they did not discuss the statistical significance of their data. Reinstein *et al.* (1985) found that rats fed on a high tyrosine diet had smaller plasma corticosterone and behavioural responses to electric shocks, presumably indicating increased tolerance to stress.

Idazoxan is a highly selective α_2-adrenoceptor antagonist; it has minimal activity at α_1-adrenoceptors and no significant activity at β-adrenoceptors, opiate, histamine, acetylcholine or 5-hydroxytryptamine receptors (Doxey *et al.*, 1983). Intravenous injections of idazoxan in the rat cause significant presynaptic α_2-adrenoceptor antagonist effects in the central nervous system (Freedman & Aghajanian, 1984), but the drug has no effect on the turnover of dopamine, in contrast to the previously used α_2-adrenoceptor antagonist, yohimbine (Walter *et al.*, 1984). In the dose used in this study, idazoxan

Table 1 The effects of placebo, idazoxan (0.1 mg kg^{-1}), tyrosine 500 mg kg^{-1} and the combination of tyrosine and idazoxan on plasma neutral amino acid concentrations (μmol l^{-1}) and the ratio of the concentration of tyrosine to the sum of the concentrations of the other large neutral amino acids (LNAA)

	Tyr	*Val*	*Isoleu*	*Leu*	*Phe*	*Tryp*	*Tyr/LNAA*
Placebo							
−15 min	59.4 ± 4.2	251.4 ± 19.4	65.2 ± 3.6	135.6 ± 16.1	62.6 ± 6.2	125.8 ± 18.8	0.09 ± 0.009
105 min	51.8 ± 5.1	202.6 ± 26.4	72.0 ± 14.3	133.4 ± 18.5	61.4 ± 11.0	109.0 ± 16.2	0.09 ± 0.01
Tyrosine							
−15 min	50.2 ± 2.8	226.6 ± 13.6	53.4 ± 6.2	126.6 ± 8.5	54.8 ± 2.1	134.4 ± 19.2	0.08 ± 0.006
105 min	467.6 ± 135	238.4 ± 19.7	58.0 ± 4.2	116.4 ± 13.6	59.4 ± 2.5	134.8 ± 24.4	0.77 ± 0.23
Idazoxan							
−15 min	55.6 ± 4.6	239.4 ± 21.2	71.4 ± 13.0	129.4 ± 17.4	56.4 ± 3.1	112.8 ± 19.5	0.09 ± 0.01
105 min	48.0 ± 4.9	207.2 ± 18.8	67.8 ± 14.6	135.8 ± 24.8	50.2 ± 4.8	113.8 ± 9.7	0.08 ± 0.006
Tyrosine + idazoxan							
−15 min	55.4 ± 5.0	194.0 ± 15.9	55.8 ± 4.8	103.4 ± 11.5	56.2 ± 2.3	123.4 ± 20.4	0.10 ± 0.01
105 min	368.4 ± 67.1	190.6 ± 9.5	41.8 ± 4.1	89.0 ± 12.3	50.2 ± 3.4	128.2 ± 15.9	0.73 ± 0.13

Data shown are means ± s.e.mean.

abolishes the sedative action of the α_2-adrenoceptor agonist clonidine in man, indicating that it exerts significant α_2-adrenoceptor antagonist activity in the central nervous system (Clifford *et al.*, 1985). When administered alone in this study, idazoxan caused a small increase in systolic blood pressure, confirming that it was administered in a biologically effective dose, but the drug had no effect on plasma noradrenaline concentrations, presumably because the latter represents a spill over effect of noradrenaline released from the peripheral sympathetic neurones. The effectiveness of an α_2-adrenoceptor antagonist is partly dependent upon the concentration of noradrenaline at the receptor; as noradrenaline release from sympathetic neurones is minimal under basal circumstances, this may explain the relatively small changes in sympathetic activity observed in this study.

We postulated that the lack of effect of tyrosine on adrenocortical activity might be due to activation of one of the negative feedback processes that control the rate of conversion of tyrosine to catecholamines and their subsequent release. In this study, we have examined the role of the presynaptic α_2-adrenoceptors, which inhibit the release of noradrenaline from the central and peripheral noradrenergic neurones. Following pretreatment with idazoxan, the administration of tyrosine caused a significant increase in plasma noradrenaline concentrations, indicating activation of the peripheral sympathetic nervous system, and of plasma ACTH concentrations. On the basis of our previous findings, we suggest that the increase in the activity of the hypothalamo-pituitary-adrenal axis represents an increase in noradrenaline turnover in the central nervous system. We have previously found that activation of peripheral adrenoceptors with a hydrophilic adrenoceptor agonist that does not cross the blood-brain barrier causes no increase in pituitary-adrenal activity (Al-Damluji *et al.*, 1987a). The increase in plasma ACTH concentrations occurred later than that of plasma noradrenaline, presumably reflecting the delay in the passage of the drugs across the blood brain barrier. Our data therefore demonstrate that, in the presence of α_2-adrenoceptor blockade, the administration of tyrosine results in an increase in the rate of release of noradrenaline and secretion of ACTH.

The large neutral amino acids (LNAA) share a common, competitive mechanism for transport across the blood-brain barrier and a similar mechanism has been proposed for the entry of tyrosine into neurones (Partridge & Oldendorf, 1977; Morre & Wurtman, 1981). In the rat, manipulations of plasma amino acid concentrations have demonstrated that brain tyrosine concentrations are directly related to the ratio of the plasma concentration of tyrosine to the sum of the concentrations of the other LNAA (Fernstrom & Faller, 1978; Agharanya & Wurtman, 1982). On the basis of the evidence in experimental animals, the increment in this ratio in our study can be expected to have increased the uptake of tyrosine across the blood brain barrier and into the neurones.

Under basal conditions, the administration of tyrosine to experimental animals has no effect on noradrenaline turnover (Gibson & Wurtman, 1978; Reinstein *et al.*, 1984) and this is compatible with the findings in this study. In stressed animals, the increased noradrenergic neuronal discharge rate results in increased activity of the tyrosine hydroxylase enzyme (Gordon *et al.*, 1966), and the administration of tyrosine under those conditions results in increased noradrenaline turnover (Gibson & Wurtman, 1978; Reinstein *et al.*, 1984). This may explain the findings by some investigators of increased urinary catecholamine excretion rates following the administration of tyrosine to ambulant human subjects, in whom sympathoadrenal discharge is presumably increased in response to the upright posture (Agharanya *et al.*, 1981). Rasmussen *et al.* (1983) found a small, transient increase in plasma catecholamine concentrations following the administration of tyrosine in the afternoon in five out of six subjects, and it is possible that this may be related to the circadian increase in catecholamine turnover in the afternoon (Wilkes *et al.*, 1981).

The lack of effect of tyrosine on noradrenaline turnover under basal conditions may be due, partly, to feedback inhibition of the tyrosine hydroxylase enzyme (which converts tyrosine to dihydroxyphenylalanine, L-DOPA) by the products of the catecholamine synthesis pathway, as the administration of tyrosine to animals pretreated with a DOPA decarboxylase inhibitor results in higher brain concentrations of L-DOPA (Wurtman *et al.*, 1974; Carlsson & Lindqvist, 1978). We have demonstrated, in the present study, that the lack of effect of tyrosine under basal conditions is partly due to the activation of α_2-adrenoceptors, which inhibit noradrenaline release. Whether idazoxan in the doses used in man has an effect on the activity of the tyrosine hydroxylase enzyme will be the subject of further investigations. David *et al.* (1974) found that in mice pretreated with a monoamine oxidase inhibitor, a large proportion of an administered dose of tyrosine was converted to tyramine by decarboxylation of the amino acid, but their data were not confirmed by other investigators (Tallman *et al.*, 1976). Release of noradrenaline by tyramine is an unlikely explanation for our findings, as tyrosine on its own had no effect on plasma noradrenaline concentrations. Conversion of tyrosine to noradrenaline seems the most likely explanation for our findings, although the experimental evidence regarding this is not yet available.

The stimulation of prolactin secretion by tyrosine confirms the findings of Ishizuka and his colleagues (1983). Our results show that the stimulant effect of tyrosine on prolactin secretion is dose-dependent in the range 50–250 mg kg^{-1}, and that tyrosine also stimulates TSH secretion. The mechanism of the stimulant effect of tyrosine on these two hormones is unknown, but it is possible that tyrosine or one of its derivatives may stimulate the secretion of the thyrotrophin releasing hormone (TRH) or inhibit or antagonize the action of hypothalamic dopamine, which would result in an increase in prolactin and TSH secretion. The apparently more potent action of tyrosine in stimulating prolactin than TSH secretion is compatible with dopaminergic inhibition or antagonism, as dopamine antagonists have a greater effect on prolactin than TSH secretion in man (Scanlon *et al.*, 1979). Interestingly, serum prolactin concentrations began to fall while plasma tyrosine concentrations were still high. This may be due to the known stimulant effect of hyperprolactinaemia on hypothalamic dopamine turnover (Hokfelt & Fuxe, 1972).

The combination of tyrosine and idazoxan caused no subjective side effects, sensations or evidence of behavioural arousal, confirming that the subjective sensations associated with the higher doses of the α_1-adrenoceptor agonist methoxamine were unlikely to be responsible for the activation of the hypothalamo-pituitary adrenal axis (Al-Damluji *et al.*, 1987a).

In summary, it is possible to activate endogenous catecholamine systems using the combination of a catecholamine precursor, tyrosine, and an α_2-adrenoceptor antagonist. This results in an increase in the activity of the hypothalamo-pituitary adrenal axis.

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